JOURNAL OF CLINICAL MICROBIOLOGY, July 1992, p. 1654–1660
0095-1137/92/071654-07$02.00/0

Vol. 30, No. 7

Detection of *Staphylococcus aureus* by Polymerase Chain Reaction Amplification of the *nuc* Gene

ODD G. BRAKSTAD,[1,2]* KJETILL AASBAKK,[2] AND JOHAN A. MAELAND[2]

Applied Chemistry Division, SINTEF, N-7034 Trondheim,[1] and Department of Microbiology, Faculty of Medicine, University of Trondheim, N-7006 Trondheim,[2] Norway

Received 28 October 1991/Accepted 2 April 1992

Synthetic oligonucleotide primers of 21 and 24 bases, respectively, were used in the polymerase chain reaction (PCR) to amplify a sequence of the *nuc* gene, which encodes the thermostable nuclease of *Staphylococcus aureus*. A DNA fragment of approximately 270 bp was amplified from lysed *S. aureus* cells or isolated DNA. The PCR product was detected by agarose gel electrophoresis or Southern blot analysis by using a 33-mer internal *nuc* gene hybridization probe. With *S. aureus* cells the lower detection limit was <10 CFU, and with the isolated target the lower detection limit was 0.69 pg of DNA. The primers recognized 90 of 90 reference or clinical *S. aureus* strains. Amplification was not recorded when 80 strains representing 16 other staphylococcal species were tested or when 20 strains representing 9 different nonstaphylococcal species were tested. Some of the non-*S. aureus* staphylococci produced thermostable nucleases but were PCR negative. The PCR product was generated when in vitro-cultured *S. aureus* was used to prepare simulated clinical specimens of blood, urine, cerebrospinal fluid, or synovial fluid. No PCR product was generated when the sterile body fluids were tested. However, the sensitivity of the PCR was reduced when *S. aureus* in blood or urine was tested in comparison with that when bacteria in saline were tested. With the bacteria in blood, the detection limit of the PCR was 10^3 CFU. A positive PCR result was recorded when a limited number of clinical samples from wounds verified to be infected with *S. aureus* were tested, while the PCR product was not detected in materials from infections caused by other bacteria. Generation of PCR products was not affected by exposure of *S. aureus* to bactericidal agents, including cloxacillin and gentamicin, prior to testing, but was affected by exposure to UV radiation. The PCR for amplification of the *nuc* gene has potential for the rapid diagnosis of *S. aureus* infections by direct testing of clinical specimens, including specimens from patients with ongoing antimicrobial therapy.

Staphylococcus aureus is the cause of serious infections in humans, including endocarditis, deep-seated abscesses, and osteomyelitis. Usually, the etiological diagnosis is dependent on isolation of the bacterium from the focus of infection or in blood cultures. In some cases, access to the focus may be difficult or dangerous or cultural confirmation may be hampered by ongoing antimicrobial therapy. Also, serological assays for *S. aureus* infections are of limited value because of insufficient diagnostic sensitivity and specificity (30), although promising results have been reported recently (2, 9, 30, 31). Consequently, it would be desirable to find methods which could supplement the cultural and serological methods, notably, alternative methods which could secure a rapid diagnosis of *S. aureus* infection. This purpose may be achieved by techniques which enable detection of *S. aureus* nucleic acids in clinical specimens.

S. aureus strains produce an extracellular thermostable nuclease (thermonuclease [TNase]) with a frequency similar to that at which they produce coagulase (18). The TNase is a protein with a molecular mass of 17,000 Da (26). It is an endonuclease, degrading both DNA and RNA, and the enzymatic activity can resist 100°C for at least 1 h (14). The TNase protein has been well characterized (26), and its gene, the *nuc* gene, has been cloned and sequenced (11). An enzymatic test for TNase production is used in many laboratories for the identification of *S. aureus* isolates (13). However, TNase activity is not specific for *S. aureus* (6). Recently, we developed a monoclonal antibody-based sandwich enzyme-linked immunosorbent assay for detection of the *S. aureus* TNase and obtained results which indicated specificity for *S. aureus* (1). These results accord with the supposition that the *S. aureus* TNase has species-specific sequences. This was supported by the findings of Liebl et al. (17), who used a 518-bp fragment of the cloned *S. aureus* TNase gene which specifically recognized *S. aureus* strains in a membrane-based DNA hybridization test.

There are numerous applications of the polymerase chain reaction (PCR) in the diagnosis of infectious diseases (24). The sensitivity of the PCR method enables the direct detection of low concentrations of bacteria or bacterial products in clinical materials (3, 5, 7, 27–29, 32). In the present study, we took advantage of the apparently unique sequences of the *S. aureus* TNase and applied the PCR for the amplification of a sequence of the *nuc* gene by using two primers that targeted this gene.

MATERIALS AND METHODS

Strains and culture conditions. The *S. aureus* Wood 46 strain was received from P. Oeding, Bergen, Norway. *Staphylococcus intermedius*, *Staphylococcus hyicus*, and *Staphylococcus schleiferi* strains were kindly delivered by T. Wadström, Lund, Sweden. American Type Culture Collection (ATCC), Rockville, Md., and Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSM), Braunschweig, Germany, strains were purchased from those organizations. All clinical strains were isolated in our laboratory. Clinical staphylococcal and micrococcal strains were identified biochemically by the automated API Staph system (API Systems S.A., Montalieu Vercieu, France), the Staphylase test (Oxoid Diagnostic Reagents, Basingstoke, United King-

* Corresponding author.

dom) and the TNase test as described by Lachica et al. (13). Nonstaphylococcal clinical strains were identified by the appropriate API systems used routinely in our laboratory. Strains were preserved in Stuart transport medium at −80°C. Single colonies of isolates were cultured on blood agar plates or were inoculated into 1% (wt/vol) glucose broth and incubated at 37°C for 18 h.

Oligonucleotide probes. Primers were selected on the basis of the published nucleotide sequence of the 966-bp *nuc* gene derived from the *S. aureus* Foggi strain (23). Several primers were synthesized on a System 200 A DNA synthesizer (Beckman Instruments Inc., Palo Alto, Calif.), vacuum dried, and stored at −20°C in double-distilled water. Two primers were selected after checking for self-complementarity. The sequences of the two primers of 21 and 24 bases, respectively, were 5′-GCGATTGATGGTGATACGGTT-3′ (primer 1) and 5′-AGCCAAGCCTTGACGAACTAAAGC-3′ (primer 2). The primers were located within the 447-bp *nuc* A gene, which encodes the nuclease A (23); primer 1 was between nucleotides +48 and +70, and primer 2 was between nucleotides +303 and +328. The primers were used without further purification.

For hybridization studies, a 33-mer probe with the sequence 5′-GGTGTAGAGAAATATGGTCCTGAAGCAAG TGCA-3′ was used; the sequence corresponds to the nucleotides between positions +147 and +181 of the *nuc* A gene (23).

Preparation of bacteria for PCR. Broth cultures of bacteria were diluted in presterilized saline prior to lysis and amplification by PCR. For measuring the sensitivity of the PCR, 10-fold serial dilutions (10^5 to 0 bacteria per ml in saline) were tested. Specificity studies were performed by testing various bacteria ($\approx 10^3$ bacteria per ml). In these experiments, 100 μl of the diluted suspension (≈100 bacteria) was pelleted at 12,000 × *g* for 15 min in a Biofuge (Heraeus Sepatech GmbH, Osterode, Germany). The pelleted bacteria were resuspended by vortex mixing and lysed in 100 μl of lysis buffer (20 mM Tris-HCl [pH 8.3], 50 mM KCl, 1.5 mM $MgCl_2$, 0.50% [vol/vol] Tween 20, 0.45% [vol/vol] Nonidet P-40, 0.01% [wt/vol] gelatin, and 60 μg of proteinase K per ml) at 55°C for 1 h. The samples were then heated at 95°C for 5 min.

For the amplification of in vitro-cultured *S. aureus* in simulated clinical specimens, $\approx 10^4$ *S. aureus* cells were added to 1 ml of culture-negative blood (whole blood in EDTA), urine, cerebrospinal fluid, or synovial fluid. Blood samples (100 μl) were mixed with 0.9 ml of 1× PCR buffer (20 mM Tris-HCl [pH 8.3], 1.5 mM $MgCl_2$) which contained 5% (wt/vol) saponin (Sigma Chemical Co., St. Louis, Mo.), incubated at 20°C for 10 min, and centrifuged at 2,000 × *g* for 5 min. The supernatants (1 ml) were pelleted (12,000 × *g*; 15 min), the pellets were resuspended in 1× PCR buffer and centrifuged (12,000 × *g*; 15 min), and the final pellets were suspended in 100 μl of lysis buffer. The cells were then lysed as described above. Other body fluids (100 μl) were diluted in 0.9 ml of 1× PCR buffer without saponin, pelleted, washed, and suspended in the lysis buffer as described above for the blood samples.

Clinical specimens on cotton swabs from wounds verified to be infected with *S. aureus* were examined. The swabs were agitated on a whirl mixer in 5 ml of sterile saline, and 100 μl was centrifuged (12,000 × *g*; 15 min). The pelleted material was suspended in 100 μl of lysis buffer and was treated as described above.

PCR amplification was performed after exposure of *S. aureus* (10^7 cells per ml) to cloxacillin (2.5 μg/ml), gentamicin (10 μg/ml), or 0.5% (vol/vol) formaldehyde at 22°C for 120 min or after exposure of the bacteria to UV radiation under a photopolymerization unit (Bio-Rad, Richmond, Calif.) for 15 min. Bacteria in pure saline (10^7/ml) and bacteria fixed in 50% (vol/vol) ethanol were used as controls. Approximately 100 cells were then pelleted and lysed as described above.

PCR amplification. The PCR amplification was performed in a thermal cycler (Coy Laboratory Products Inc., Ann Arbor, Mich.) by using a recombinant *Taq* DNA polymerase (AmpliTaq, Perkin-Elmer Cetus Corp., Norwalk, Conn.). The reaction mixture consisted of 50 μl of lysate, 10 μl of 10× PCR amplification buffer (200 mM Tris-HCl [pH 8.3], 500 mM KCl, 15 mM $MgCl_2$, 0.1% [wt/vol] gelatin, 0.5% Tween 20), 2.0 μl of each primer (20 μM stock solution), 10 μl of the deoxynucleoside triphosphates (1 mM each in stock solution), 0.4 μl of AmpliTaq (5 U/μl of stock solution), and 25.6 μl of double-distilled water. Mineral oil (50 μl) was added to the mixtures to inhibit evaporation. A total of 37 PCR cycles were run under the following conditions: DNA denaturation at 94°C for 1 min, primer annealing at 55°C for 0.5 min, and DNA extension at 72°C for 1.5 min. After the final cycle, the reaction was terminated by keeping it at 72°C for 3.5 min. The PCR products were stored in the cycler at 4°C until they were collected.

Agarose gel electrophoresis. The PCR-amplified samples were analyzed by agarose gel electrophoresis by using a horizontal 1.3% (wt/vol) agarose gel in 1× TBE buffer (pH 8.3; 0.09 M Tris, 0.09 M boric acid, 2.0 mM EDTA) and with 0.003% (wt/vol) ethidium bromide incorporated for DNA staining. Undiluted PCR products (30 μl) were mixed with a sample buffer (10 μl) of 0.4× TBE, 50% (vol/vol) glycerol, and 0.025% (wt/vol) bromphenol blue; and 25 μl of sample was applied to each well. Gels were run in 1× TBE buffer at 150 V for 2 h. The PCR products were visualized and photographed on a transilluminator (Sigma). A pBR322 *Hae*III DNA digest (Sigma) was used as a DNA marker.

Southern blot analysis. PCR products were separated on a 1.3% agarose gel and transferred to a Zeta Probe blotting membrane (Bio-Rad) essentially as described by Sambrook et al. (22), but the depurination step was omitted. The membrane was fixed under UV light on a transilluminator (5 min), prehybridized for 1 h at 51°C in 40 ml of hybridization solution (6× SSC [0.9 M NaCl, 0.09 M sodium citrate; pH 7.0], 0.5% [wt/vol] sodium dodecyl sulfate [SDS], and 10× Denhardt's solution [0.25% Ficoll 400, 0.25% polyvinylpyrrolidone, 0.25% bovine serum albumin]), and hybridized for 3 h at 52°C in hybridization solution with 10 pmol of the 33-mer DNA probe. The DNA probe was labeled with [γ-^{32}P]ATP (Amersham Life Science Products, Amersham, Buckinghamshire, United Kingdom) by using T4 polynucleotide kinase (22). The membrane was washed in 0.1× SSC–0.1% SDS for 2 h at 45°C and exposed to Hyperfilm-MP (Amersham) for 1 h at −80°C for detection of hybridization.

DNA purification. *S. aureus* DNA was purified as described by Wilson (36), except that lysostaphin (150 μl/ml; Sigma) replaced lysozyme in the procedure.

Other methods. Total numbers of cells were counted by epifluorescence microscopy after targeting cellular DNA with 0.2 μg of the fluorochrome 4′,6-diamino-2-phenylindole (Sigma) (21) per ml. Viable cells were counted as CFU by triplicate plating of samples on blood agar and counting the colonies after incubation at 37°C for 20 h.

The purity of DNA samples were assessed by determination of the optical density at 260 nm/optical density at 280 nm

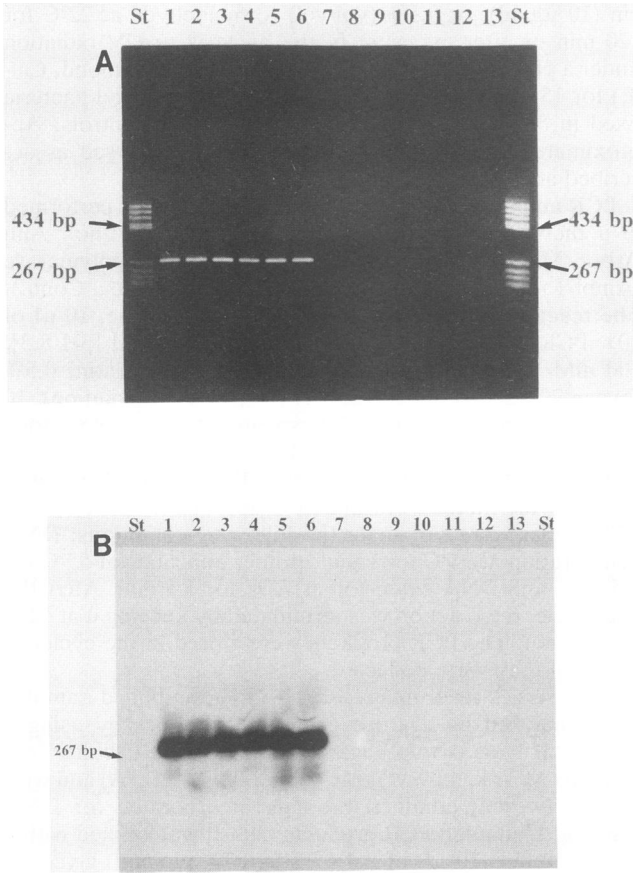

FIG. 1. Gel electrophoresis (A) and Southern blot analysis (B) of PCR products amplified from approximately 100 cells of *S. aureus* Foggi (lane 1), Wood 46 (lane 2), V8 (lane 3), M^- (lane 4), Cowan 1 (lane 5), and ATCC 25923 (lane 6) and the non-*S. aureus* staphylococci *S. epidermidis* KS 426 (lane 7), *S. saprophyticus* DSM 20229 (lane 8), *S. hominis* DSM 20328 (lane 9), *S. hyicus* DSM 20459 (lane 10), *S. intermedius* DSM 20373 (lane 11), *S. schleiferi* DSM 4807 (lane 12), and *S. cohnii* DSM 20260 (lane 13). The *Hae*III-cleaved pBR322 marker is shown in lanes St.

ratio, and DNA concentrations were estimated by measuring the optical density at 260 nm (4).

RESULTS

The primer set synthesized for this study allowed PCR amplification of a gene fragment when *S. aureus* strains were lysed and amplified by 35, 37, or 40 cycles. We found that 37 cycles was optimal. Increases in the annealing time from 0.5 to 2 min and the primer extension time from 1.5 to 2 min did not increase the sensitivity of the method. Figure 1 shows the results obtained when some reference strains of *S. aureus* and non-*S. aureus* staphylococci were tested in the PCR. The *S. aureus* strains were amplified, while the non-*S. aureus* strains were not. The PCR product appeared as a single DNA band with a size close to that of the 267-bp band of the pBR322 *Hae*III DNA digest (Fig. 1A). This size is close to the expected size of 279 bp for the PCR product. Repeat testing of some of the strains that we examined showed the same results. A 33-mer DNA probe corresponding to a *nuc* gene sequence located between the sites of the two primers hybridized to the PCR products by Southern blot analysis (Fig. 1B). This finding confirmed the *nuc* gene origin of the amplified products.

Sensitivity. The lower limit for detection of *S. aureus* bacterial cells or isolated DNA by PCR was examined. A suspension of *S. aureus* Foggi was diluted in saline, and the bacteria were counted by epifluorescence microscopy and CFU determination. Amplification which resulted in detectable levels of PCR product was achieved when a minimum of 6 CFU of *S. aureus* cells were lysed; this corresponded to 14 cells determined by epifluorescence microscopy (Fig. 2A). By repeat testing of viable *S. aureus* cells, the detection limit ranged from 5 to 20 CFU. Similar detection limits were observed when other *S. aureus* strains (Wood 46 and V8) were examined. DNA from *S. aureus* Foggi was isolated, serially diluted in saline, and used as a template. The results show (Fig. 2B) that 0.69 pg of purified DNA in the reaction mixture was the minimum needed to obtain a detectable PCR product.

Specificity. A total of 7 reference strains of *S. aureus* and 27 reference strains of other staphylococci were used as

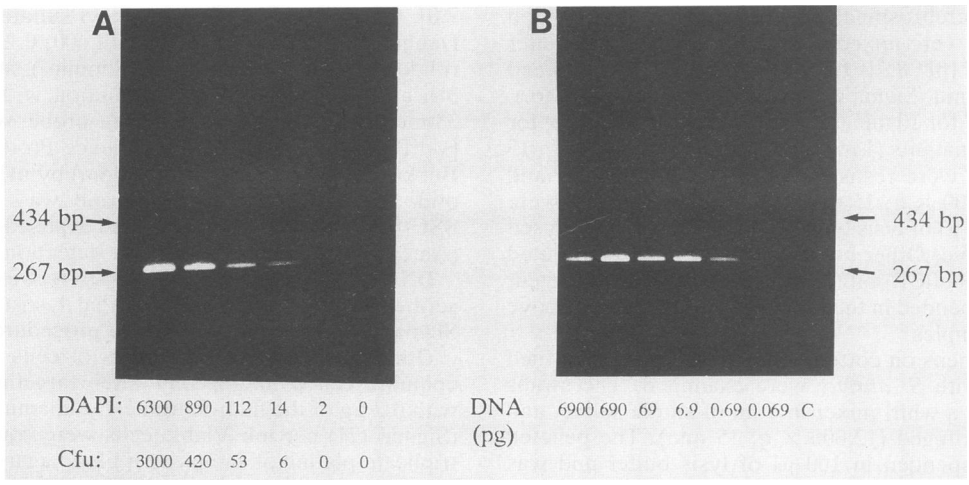

FIG. 2. Gel electrophoresis of PCR products amplified from serial dilutions of *S. aureus* Foggi cells (A) and purified *S. aureus* DNA (B). (A) Total counts of bacterial cells (4′,6-diamino-2-phenylindole [DAPI]) and CFU are indicated at the bottom of the gel; (B) the DNA content in each sample is indicated at the bottom of the gel. The positions of two pBR322 marker bands are indicated.

TABLE 1. Staphylococcal reference strains tested by the enzymatic TNase agar method and PCR for amplification of a *nuc* gene fragment

Staphylococcal strains	TNase activity[a]	PCR[b]
S. aureus Foggi	+	+
S. aureus Wood 46	+	+
S. aureus Cowan 1	+	+
S. aureus V8	+	+
S. aureus M$^-$	+	+
S. aureus ATCC 25923	+	+
S. aureus ATCC 21027	+	+
S. epidermidis KS 426	−	−
S. epidermidis I-1478 (+)	−	−
S. saprophyticus DSM 20229	−	−
S. hominis DSM 20328	−	−
S. haemolyticus DSM 20263	−	−
S. cohnii DSM 20260	−	−
S. intermedius DSM 20373	+	−
S. intermedius KLM87-1127	+	−
S. intermedius KLM87-931	+	−
S. intermedius KLM87-1236	+	−
S. intermedius KLM87-992	+	−
S. intermedius KLM87-1167	−	−
S. hyicus DSM 20459	+	−
S. hyicus 2368	+	−
S. hyicus 842A	+	−
S. hyicus A2869C	+	−
S. hyicus 9390	+	−
S. schleiferi DSM 4807	+	−
S. schleiferi 890189	+	−
S. schleiferi G 67-88	−	−
S. schleiferi N 880033	+	−
S. schleiferi G 22-89	+	−
S. capitis DSM 20326	−	−
S. sciuri DSM 20345	−	−
S. auricularis DSM 20609	−	−
S. simulans DSM 20322	−	−
S. lugdunensis DSM 4804	−	−

[a] A positive test result (+) was defined as distinct pink zones with diameters of >10 mm (13).

[b] A single band of ~270 bp was defined as a positive test result (+).

templates for the primers, each with ≈100 bacterial cells in the PCR mixture. All of the *S. aureus* strains were amplified and were thus recognized by the primers, but none of the other staphylococci were amplified or recognized by the primers (Table 1). The enzymatic test for TNase activity was positive with all of the *S. aureus* strains and with 14 of the non-*S. aureus* strains, with all of the non-*S. aureus* strains belonging to the species *S. intermedius*, *S. hyicus*, or *S. schleiferi* (Table 1). The specificity of the primer set was also examined by testing a total of 156 clinical isolates of various bacteria, including 83 *S. aureus* strains (Table 2). Only the *S. aureus* strains were recognized by the primer set and amplified. With all of the *S. aureus* isolates examined, a PCR product of approximately 270 bp was generated. Of the non-*S. aureus* clinical staphylococcal strains (n = 53), three *S. schleiferi* strains showed TNase activity but were PCR negative (Table 2).

PCR of body fluids. For PCR analysis of clinical specimens, biological materials should not interfere with the reaction. Culture-negative whole blood, urine, synovial fluid, and cerebrospinal fluid were tested with and without in vitro-cultured *S. aureus* Foggi bacteria added (≈1,000 bacteria in the reaction mixture) (Fig. 3). All of the samples which contained bacteria were amplified. When tested with no bacteria added, none of the biological fluids gave rise to amplification products. The sensitivity of the PCR was examined with *S. aureus* serially diluted in the various body fluids. With the bacteria in the cerebrospinal or synovial fluid, the lower detection limit ranged from 10 to 20 CFU, which was similar to the limit recorded with bacteria in saline. However, with *S. aureus* in urine, the lower detection limit was 100 CFU, and in whole blood it was 1,000 CFU.

A total of 29 clinical samples collected on cotton swabs from patients with verified wound infections caused by *S. aureus* (n = 19) or other bacteria (n = 10) were tested by the PCR. The results for some of these samples are shown in Fig. 4. All of the samples which grew *S. aureus* gave rise to a positive PCR result with bands of the expected size, but none of the samples which grew other bacteria gave rise to a positive PCR result. The intensities of the bands varied. Weak bands were recorded from samples with a low number of *S. aureus* or materials admixed with large quantities of blood (data not shown). For two of the non-*S. aureus* samples (*Klebsiella pneumoniae* and *Escherichia coli*), weak bands (≈500 bp) could be visualized (Fig. 4, lane 11).

PCR with bacteria exposed to antimicrobial agents. To analyze whether *S. aureus* Foggi cells exposed to bactericidal agents were amplified, the bacteria (10^7 bacterial cells) were exposed to cloxacillin or gentamicin at concentrations above the MICs for the bacteria, UV irradiation, formaldehyde, or ethanol. Exposure to antimicrobial agents resulted in a >99% CFU reduction; exposure to the other agents resulted in 100% CFU reduction. The irradiation totally inhibited the PCR amplification when ≈100 bacterial cells were tested. The other agents had no effect on the gene product observed after electrophoresis.

DISCUSSION

Nucleic acid amplification by PCR has applications in many fields of biology and medicine, including the detection of viruses, bacteria, and other infectious agents (for a review and references, see reference 24). In the present study, we synthesized an oligonucleotide primer set which recognized sequences of the *S. aureus nuc* gene, which encodes the TNase produced by these bacteria. This strategy was chosen since earlier data obtained by using polyclonal (6, 15) or monoclonal (1) antibodies to detect the *S. aureus* TNase indicated that this protein has species-specific sequences and that DNA hybridization-based methods corroborated the assumption that this protein is species specific (17). On the other hand, some non-*S. aureus* staphylococci, streptococci, and possibly other bacteria (6) may produce nucleases with enzymatic activity that mimics that of the *S. aureus* TNase.

A simple method of lysing the bacteria was applied in this study. The primer set determined the generation of a PCR product of approximately 270 bp, which is close to the expected gene fragment size of 279 bp (23). A 33-mer DNA probe, corresponding to an internal sequence of the 270-bp segment, hybridized with the PCR products. This confirmed that the product is identical to a sequence of the *S. aureus nuc* gene.

Strict precautions must be taken to avoid false-positive PCR results because of amplification of contaminating DNA (12). Early in our experiments, we were confronted with this problem; we eliminated contaminating DNA by irradiating all the equipment used in the preamplification steps with UV light, in accordance with the precautions recommended by others (20) and with our own results, which confirmed that

TABLE 2. Clinical isolates of *S. aureus* and various other bacteria tested by the enzymatic TNase agar method and PCR for amplification of a *nuc* gene fragment

Bacterial species (no. of isolates)	Source of isolate	No. of strains positive for:	
		TNase activity[a]	PCR amplification[b]
S. aureus (47)	Blood culture	47	47
S. aureus (6)	Urine	6	6
S. aureus (5)	Secretion	5	5
S. aureus (23)	Abcess	23	23
S. aureus (2)	Synovial fluid	2	2
S. epidermidis (26)	Blood culture	0	0
S. hominis (7)	Blood culture	0	0
S. haemolyticus (8)	Blood culture	0	0
S. warneri (3)	Blood culture	0	0
S. xylosis (2)	Blood culture	0	0
S. caprae (1)	Blood culture	0	0
S. schleiferi (3)	Blood culture	3	0
S. lugdunensis (3)	Blood culture	0	0
Streptococcus spp. (3)	Blood culture	0	0
Enterococcus sp. (1)	Blood culture	0	0
Micrococcus spp. (4)	Blood culture	0	0
Escherichia coli (4)	Blood culture	0	0
Enterobacter cloacae (2)	Blood culture	0	0
Klebsiella pneumoniae (3)	Blood culture	0	0
Klebsiella oxytoca (1)	Blood culture	0	0
Proteus mirabilis (1)	Blood culture	0	0
Candida sp. (1)	Blood culture	0	0

[a] For definition of positive test results, see footnote *a* of Table 1.
[b] For definition of positive tests, see footnote *b* of Table 1.

UV irradiation of *S. aureus* eliminates the generation of a PCR product.

The *nuc* PCR detected <20 viable *S. aureus* cells or correspondingly low levels (0.69 pg) of extracted DNA in saline. The sensitivity accords with that described for PCR with other bacteria, being between 1 and 20 CFU (16, 19, 29, 32) or between 1 and 100 pg for DNA extracted from *S. aureus* (8, 35). An increase in sensitivity may be achieved by nested PCR amplification (35).

The *nuc* primer set recognized all staphylococci identified as *S. aureus* by conventional methods, but it did not recognize the other bacteria tested. These results substantiate those obtained by other methodological approaches (1, 6, 15, 17), which have suggested that the *nuc* gene and its product have sequences which, on the one hand, are found in all *S. aureus* isolates and, on the other hand, are unique to bacteria of that species.

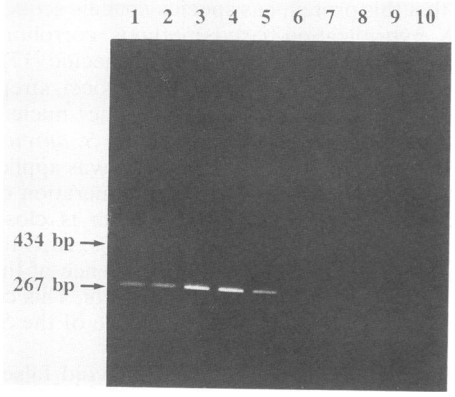

FIG. 3. Gel electrophoresis of PCR products generated with approximately 1,000 cells of *S. aureus* Foggi amplified in culture-negative whole blood (lane 1), urine (lane 2), synovial fluid (lane 3), cerebrospinal fluid (lane 4), and saline (lane 5). Lanes 6 to 10, the same fluids in lanes 1 to 5, respectively, without bacteria added. The positions of two pBR322 marker bands are indicated on the left.

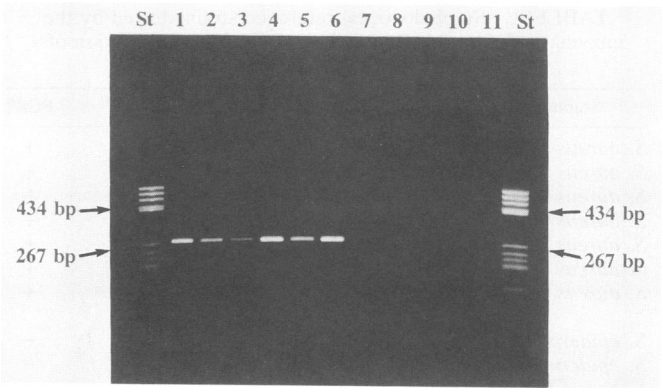

FIG. 4. Gel electrophoresis of PCR products generated by direct testing of clinical specimens collected on cotton swabs from wounds infected with *S. aureus* (lanes 1 to 6), *S. epidermidis* (lanes 7 to 9), *Enterobacter cloacae* (lane 10), and *Klebsiella pneumoniae* (lane 11). The *Hae*III-cleaved pBR322 marker is shown in lanes St.

Previously, investigators have described PCR amplification of the *S. aureus* gene encoding for methicillin resistance (25) and of genes encoding toxins, such as the exfoliative toxins, toxic shock syndrome toxins, and enterotoxins (8, 35), which are produced by many *S. aureus* isolates. Recently, PCR amplification of the *nuc* gene was reported by Wilson et al. (35), who used a primer set different from that used in the present study. Those investigators amplified a 450-bp segment of the gene. However, very few data of this amplification were described (35). The combination of the *nuc* PCR for species identification of *S. aureus* and PCRs for the identification of genes encoding defined phenotypic characteristics of the bacteria is therefore possible.

DNA amplification by PCR has applicability in the diagnosis of infectious diseases, provided that the tests have adequate sensitivity and specificity. For instance, this method was equivalent to culture with respect to the detection of *Haemophilus influenzae* in cerebrospinal fluid (29), *Chlamydia trachomatis* in various clinical specimens (3), and *Borrelia burgdorferi* in clinical material from experimentally infected animals (16). The sterile body fluids which we tested in the *S. aureus nuc* PCR—blood, synovial fluid, urine, and cerebrospinal fluid—were negative in the test, whereas positive test results were recorded provided that *S. aureus* was added to produce simulated culture-positive clinical specimens. However, with bacteria in urine, and notably with bacteria in blood, the sensitivity of the *nuc* PCR was far lower than that with bacteria in saline. With bacteria in blood, the lower detection limit was higher than the number of CFU per unit volume of blood, which usually is found in patients with bacteremia (10). We added saponin to the blood since this is reported to lyse phagocytes and to release phagocytosed bacteria (33). By this treatment we also anticipated removal of most of the hemoglobin, which is known to interfere with the PCR (34). The reduced sensitivity of the *nuc* PCR for detection of *S. aureus* in blood may be due to residual interfering components.

All of the 19 swab specimens which contained *S. aureus* were positive by direct testing in the *nuc* PCR for the 270-bp PCR product, whereas all specimens which grew other bacteria were negative. These findings substantiate the prospects of the rapid diagnosis of *S. aureus* infections by the *nuc* PCR described in this report, for instance, when a rapid diagnosis is important in order to initiate adequate therapy immediately or when ongoing therapy interferes with the in vitro growth of the bacteria. Our data indicate that treatment with antibiotics does not interfere with the detection of the *nuc* gene as long as sufficient quantities of the target DNA sequences are still present in the clinical specimens. We observed that the quantity of the 270-bp PCR product varied with the number of *S. aureus* CFU isolated from the specimen. Lebech et al. (16) reported that extraction of DNA is needed when actual clinical specimens instead of simulated specimens are tested. This method and other techniques that may increase the sensitivity of the *nuc* PCR for the detection of *S. aureus* in clinical specimens need to be investigated further. Notably, methodological improvements are required to increase the sensitivity of the test for the rapid detection of *S. aureus* bacteremia, since this is a particularly important clinical situation for application of the test.

Also, the *nuc* PCR may be applicable for testing nonclinical samples like food or as a research tool, for instance, in studies of the distribution and fate of *S. aureus* in various organs and cells of the body.

ACKNOWLEDGMENTS

We thank Kristin Lund Borg for synthesizing the oligonucleotide primers, Per Vinge for calculations of primer self-complementarity, and Frank Karlsen for isolating DNA.

This work was supported by the Norwegian Research Council for Science and the Humanities.

REFERENCES

1. **Brakstad, O. G., and J. A. Maeland.** 1989. Generation and characterization of monoclonal antibodies against *Staphylococcus aureus*. Acta Pathol. Microbiol. Immunol. Scand. **97:**166–174.
2. **Brakstad, O. G., J. A. Maeland, and H. I. Wergeland.** 1989. Serum antibodies to a *Staphylococcus aureus* thermonuclease preparation in healthy persons and patients with bacteraemia. Serodiagn. Immunother. Infect. Dis. **3:**201–210.
3. **Claas, H. C., W. J. Melchers, I. H. de Bruijn, M. de Graf, W. C. van Dijk, J. Lindeman, and W. G. Quint.** 1990. Detection of *Chlamydia trachomatis* in clinical specimens by the polymerase chain reaction. Eur. J. Clin. Microbiol. Infect. Dis. **9:**864–868.
4. **Davis, L. G., M. D. Dibner, and J. F. Battey.** 1986. Basic methods in molecular biology, p. 327–328. Elsevier Science Publishing Co., Inc., New York.
5. **De Wit, M. Y. L., W. R. Faber, S. R. Krieg, J. T. Douglas, S. B. Lucas, N. Montreewasuwat, S. R. Pattyn, R. Hussain, J. M. Ponnighaus, R. A. Hartskeerl, and P. R. Klatser.** 1991. Application of a polymerase chain reaction for the detection of *Mycobacterium leprae* in skin tissues. J. Clin. Microbiol. **29:**906–910.
6. **Gudding, R.** 1983. Differentiation of staphylococci on the basis of nuclease properties. J. Clin. Microbiol. **18:**1098–1101.
7. **Hay, P. E., F. R. Clarke, D. Taylor-Robinson, and D. Goldmeier.** 1990. Detection of treponemal DNA in the CSF of patients with syphilis and HIV infection using the polymerase chain reaction. Genitourin. Med. **66:**428–432.
8. **Johnson, W. M., S. D. Tyler, E. P. Ewan, F. E. Ashton, D. R. Pollard, and K. R. Rozee.** 1991. Detection of genes for enterotoxins, exfoliative toxins, and toxic shock syndrome toxin 1 in *Staphylococcus aureus* by the polymerase chain reaction. J. Clin. Microbiol. **29:**426–430.
9. **Julander, I. G., M. Granström, S. Å. Hedström, and R. Möllby.** 1983. The role of antibodies against alpha-toxin and techoic acid in the diagnosis of staphylococcal infections. Infection **11:**77–83.
10. **Kiehn, T. E., and D. Armstrong.** 1990. Changes in the spectrum of organisms causing bacteremia and fungemia in immunocompromised patients due to venous access devices. Eur. J. Clin. Microbiol. Infect. Dis. **9:**869–872.
11. **Kovacevic, S., L. E. Veal, H. M. Hsiung, and J. R. Miller.** 1985. Secretion of staphylococcal nuclease by *Bacillus subtilis*. J. Bacteriol. **162:**521–528.
12. **Kwok, S., and R. Higuchi.** 1989. Avoiding false positives with PCR. Nature (London) **339:**237–238.
13. **Lachica, R. V. F., C. Genigeorgis, and P. D. Hoeprich.** 1971. Metachromic agar-diffusion methods for detecting staphylococcal nuclease activity. Appl. Microbiol. **21:**585–587.
14. **Lachica, R. V. F., P. D. Hoeprich, and H. P. Riemann.** 1972. Tolerance of staphylococcal thermonuclease to stress. Appl. Microbiol. **23:**994–997.
15. **Lachica, R. V. F., S. S. Jang, and P. D. Hoeprich.** 1979. Thermonuclease seroinhibition test for distinguishing *Staphylococcus aureus* from other coagulase-positive staphylococci. J. Clin. Microbiol. **9:**141–143.
16. **Lebech, A.-M., P. Hindersson, J. Vuust, and K. Hansen.** 1991. Comparison of in vitro culture and polymerase chain reaction for detection of *Borrelia burgdorferi* in tissue from experimentally infected animals. J. Clin. Microbiol. **29:**731–737.
17. **Liebl, W., R. Rosenstein, F. Götz, and K. H. Schleifer.** 1987. Use of a staphylococcal nuclease gene as DNA probe for *Staphylococcus aureus*. FEMS Microbiol. Lett. **44:**179–184.
18. **Madison, B. M., and V. S. Baselski.** 1983. Rapid identification of *Staphylococcus aureus* in blood cultures by thermonuclease testing. J. Clin. Microbiol. **18:**722–724.
19. **Olive, D. M.** 1989. Detection of enterotoxigenic *Escherichia coli* after polymerase chain reaction amplification with a thermostable DNA polymerase. J. Clin. Microbiol. **27:**261–265.
20. **Ou, C.-Y., J. L. Moore, and G. Schochetman.** 1991. Use of UV irradiation to reduce false positivity in polymerase chain reaction. BioTechniques **10:**442–445.
21. **Porter, K., and J. H. Paul.** 1980. The use of DAPI for identifying and counting aquatic microflora. Limnol. Oceanogr. **25:**943–948.
22. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed., p. 11.31–11.32. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
23. **Shortle, D.** 1983. A genetic system for analysis of staphylococcal nuclease. Gene **22:**181–189.
24. **Thiele, D.** 1990. The technique of polymerase chain reaction—a new diagnostic tool in microbiology and other scientific fields (review). Zentralbl. Bateriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. **273:**434–454.
25. **Tokue, Y., S. Shoji, K. Satoh, A. Watanabe, and M. Motomiya.** 1991. Detection of methicillin-resistant *Staphylococcus aureus* (MRSA) using polymerase chain reaction amplification. Tohoku J. Exp. Med. **163:**31–37.
26. **Tucker, P. W., E. E. Hazen, and F. A. Cotton.** 1978. Staphylococcal nuclease reviewed: a prototypic study in contemporary enzymology. I. Isolation, physical and enzymatic properties. Mol. Cell. Biochem. **22:**67–77.
27. **Valentine, J. L., R. R. Arthur, H. L. T. Mobley, and J. D. Dick.** 1991. Detection of *Helicobacter pylori* by using the polymerase chain reaction. J. Clin. Microbiol. **29:**689–695.
28. **Van Eys, G. J. J. M., C. Gravekamp, M. J. Gerritsen, W. Quint, M. T. E. Cornelissen, J. Ter Schegget, and W. J. Terpstra.** 1989. Detection of leptospires in urine by polymerase chain reaction. J. Clin. Microbiol. **27:**2258–2262.
29. **Van Ketel, R. J., B. De Wever, and L. Van Alphen.** 1990. Detection of *Haemophilus influenzae* in cerebrospinal fluids by polymerase chain reaction DNA amplification. J. Med. Microbiol. **33:**271–276.
30. **Verbrugh, H. A., R. D. Nelson, P. K. Peterson, B. J. Wilkinson, and R. L. Thompson.** 1983. Serology of *Staphylococcus aureus* infections using multiple antigens and serial serum samples. J. Infect. Dis. **148:**608.
31. **Verbrugh, H. A., R. Peters, W. H. F. Goessens, and M. F.**

Michel. 1986. Distinguishing complicated from uncomplicated bacteremia caused by *Staphylococcus aureus*: the value of "new" and "old" serological tests. J. Infect. Dis. **153:**109–115.

32. **Viitanen, A.-M., T. P. Arstila, R. Lahesmaa, K. Granfors, M. Skurnik, and P. Toivanen.** 1991. Application of the polymerase chain reaction and immunofluorescence techniques to the detection of bacteria in *Yersinia*-triggered reactive arthritis. Arthritis Rheum. **34:**89–96.
33. **Virji, M., and J. E. Heckels.** 1985. Role of anti-pilus antibodies in host defense against gonococcal infection studied with monoclonal anti-pilus antibodies. Infect. Immun. **49:**621–628.
34. **Warhurst, D. C., F. M. A. El Kariem, and M. A. Miles.** 1991. Simplified preparation of malarial blood samples for polymerase chain reaction. Lancet **337:**303–304.
35. **Wilson, I. G., J. E. Cooper, and A. Gilmour.** 1991. Detection of enterotoxigenic *Staphylococcus aureus* in dried skimmed milk: use of the polymerase chain reaction for amplification and detection of staphylococcal enterotoxin genes *entB* and *entC1* and the thermonuclease gene *nuc*. Appl. Environ. Microbiol. **57:**1793–1798.
36. **Wilson, K.** 1987. Preparation of genomic DNA from bacteria, p. 2.4.1–2.4.4. *In* F. M. Ausubel et al. (ed.), Current protocols in molecular biology, vol. 1. Green Publishing Associates and John Wiley & Sons, Inc., New York.